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BY

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A comparison of the "Defibrination" and "Oxalate" methods of Serum Preparation as applied to Haemorrhagic Septicaemia and Anthrax Sera together with some Analyses of Buffalo and Hill Bull Blood.

Introduction.

IN the following notes a brief account is given of some comparative experiments carried out on the preparation of Hæmorrhagic Septicæmia and Anthrax sera by two different methods, the chief object in view being to study the influence of the method on the yield and quantity of the serum. As regards yield this depends on several factors, *e.g.*, (1) the nature of the animal and (2) nature of the bleeding. The animals used in this laboratory are chiefly buffaloes and hill bulls. Formerly, each animal after immunisation was bled twice with an interval of two days between the bleedings, the first bleeding being at the rate of 6 cc. per lb. body weight and the second at 8 cc. This method, however, is now discontinued and three bleedings are taken in each series, all three being at the rate of 6 cc. per lb. body weight and with an interval of 4 days between each bleeding. It is found that more serum can be obtained from the second bleeding than from the first while the third gives the largest percentage of all. Hence in the tables which follow the bleedings have been classified into three groups according as they were the 1st, 2nd and 3rd in a series.

Defibrination method.

The blood is drawn into bottles containing a coil of copper wire, defibrinated by shaking and the defibrinated blood then centrifugalised. The advantages of the method are (1) great simplicity and (2) rapidity. On the other hand, the serum is usually of a bad colour containing an appreciable amount of hæmoglobin. When carbolised a considerable deposit settles out and on standing the colour becomes extremely dark.

Oxalate method.

The blood is drawn into bottles containing a small quantity of potassium oxalate solution, the latter being used at the rate of 10 cc. of a 10 per cent. solution to each litre of blood. The bottle is rotated to secure thorough mixing and the blood is then allowed to stand. In the case of *buffaloes*, in a few hours, the corpuscles have almost completely settled and a large proportion (50 per cent. of the blood) of the clear plasma can be syphoned off. The residue in the bottle is then centrifugalised when another 10-15 per cent. plasma is obtained.

In the case of *hill bulls*, the corpuscles settle with extreme difficulty and it is usually necessary to centrifugalise the whole of the blood. This curious difference which is of course of great practical importance will be dealt with again later in this paper. The oxalated plasma obtained in the above manner is then clotted by the addition of 10 cc. of a 12 per cent. solution of calcium chloride to each litre of plasma. An extremely tough, almost white clot forms in a few minutes. This, on separation from the sides of the vessel, rapidly contracts to a very small bulk, this process being accelerated by the addition of weights. The majority of the corpuscles having been removed before the clot forms, the expressed serum is of an extremely good appearance, being free from any red colouration, a point of some importance in this country. When carbolised, moreover, serum prepared in this way exhibits much less precipitation than that obtained by defibrination and in the limited time of observation possible, since this method was begun, does not appear to darken in colour so readily. Finally, it has been found that the yields of serum obtained by the oxalate method are, as a rule, markedly higher than by defibrination. This last factor in itself amply repays the increased time required for the oxalate method.

In the following tables are given the results obtained with hæmorrhagic septicæmia and anthrax sera by the two methods. As the tables are classified according to the nature of the bleeding the influence of this factor can also be observed.

TABLE I.

Hæmorrhagic Septicæmia. Defibrination method.

Buffaloes. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	3,800	2,850	75·0	1,900	66·7	**50·0**
2	15,800	13,150	83·4	7,300	55·5	**46·2**
3	3,000	2,450	81·7	1,250	51·0	**41·7**
4	5,900	4,250	72·2	2,900	68·2	**49·6**
5	4,700	3,250	69·1	2,350	72·3	**50·0**
6	5,700	4,900	85·9	3,100	63·3	**54·4**
7	3,400	2,800	82·3	2,000	71·4	**58·8**
8	9,300	7,800	83·9	5,050	64·7	**54·3**
TOTAL .	51,600	41,450	80·3	25,850	62·4	**50·1**

Average yields—

(1) Defibrinated blood	80·0
(2) Serum—	
(*a*) Percentage of defibrinated blood . . .	64·1
(*b*) Percentage of blood drawn	**50·6**

TABLE II.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Buffaloes. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	SERUM		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	1,300	1,000	76·9	900	90·0	**69·2**
2	1,500	1,050	70·0	950	90·5	**63·3**
3	10,200	6,400	62·7	5,030	78·6	**49·3**
4	2,900	1,700	58·6	1,400	82·4	**48·2**
5	3,400	2,300	67·6	2,100	91·3	**61·1**
6	3,200	1,800	56·2	1,550	91·6	**48·5**
7	1,800	1,100	61·1	1,000	90·9	**55·5**
TOTAL .	24,300	15,350	63·5	12,930	84·2	**53·4**

Average yields—

(1) Plasma	64·7
(2) Serum—	
(*a*) Percentage of plasma . . .	87·9
(*b*) Percentage of whole blood drawn .	**56·4**

TABLE III.

HÆMORRHAGIC SEPTICÆMIA. DEFIBRINATION METHOD.

Buffaloes. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	SERUM		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	9,900	8,200	82·8	5,170	63·0	**52·2**
2	5,000	3,850	77·0	2,400	62·3	**48·0**
3	13,700	11,200	81·7	6,950	62·0	**50·7**
4	1,300	1,050	80·8	700	66·7	**53·9**
5	3,000	2,250	75·0	1,450	64·5	**48·3**
6	5,600	4,900	87·5	3,300	67·4	**58·9**
TOTAL	38,500	31,450	81·7	19,970	63·5	**51·9**

Average yields—

(1) Defibrinated blood	81·0
(2) Serum—	
(*a*) Percentage of defibrinated blood	64·2
(*b*) Percentage of whole blood drawn	**52·0**

TABLE IV.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Buffaloes. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	Serum		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	1,500	900	60·0	830	92·2	**55·3**
2	6,300	4,200	66·7	3,950	94·0	**62·7**
3	7,300	5,350	73·3	5,100	95·3	**69·9**
4	1,600	1,100	68·7	1,000	90·9	**62·5**
5	8,300	4,500	54·3	4,200	93·3	**50·6**
6	2,900	1,850	63·8	1,650	89·2	**56·8**
7	2,900	1,800	62·2	1,650	91·6	**56·8**
TOTAL .	30,800	19,700	63·9	18,380	93·4	**59·6**

Average yields—

(1) Plasma	64·1
(2) Serum—	
(*a*) Percentage of plasma	92·3
(*b*) Percentage of whole blood drawn . . .	**59·2**

TABLE V.

HÆMORRHAGIC SEPTICÆMIA. DEFIBRINATION METHOD.

Buffaloes. *3rd Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	5,600	4,350	77·7	3,200	73·6	**57·2**
2	2,200	1,900	83·7	1,200	63·2	**54·5**
3	11,000	9,550	86·8	6,400	67·0	**58·2**
4	1,300	1,050	80·8	750	71·4	**57·7**
5	3,000	2,550	85·0	1,950	76·5	**65·0**
TOTAL .	23,100	19,400	84·0	13,500	69·6	**58·4**

Average yields—

(1) Defibrinated blood	82·8
(2) Serum—	
(*a*) Percentage of defibrinated blood	70·3
(*b*) Percentage of whole blood drawn . . .	**58·5**

TABLE VI.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Buffaloes. *3rd Bleedings.*

Series	Volume of blood drawn cc.	Volume of plasma obtained	Percentage yield of plasma	Serum		
				Volume obtained.	Percentage of plasma	Percentage of whole blood
1	10,600	6,150	58·0	6,100	99·0	**57·5**
2	1,700	1,000	58·8	970	97·0	**57·1**
3	2,700	2,100	77·8	1,900	90·5	**70·4**
4	7,600	5,200	68·4	4,750	91·4	**62·5**
5	1,300	900	69·2	850	94·4	**65·4**
6	3,300	2,300	70·0	2,050	89·1	**62·1**
7	3,400	2,600	76·4	2,350	90·4	**68·8**
8	8,300	5,250	63·2	4,800	91·4	**57·8**
9	3,200	2,300	71·9	2,050	89·1	**64·0**
TOTAL .	42,100	27,800	66·2	25,820	92·8	**61·5**

Average yields—

(1) Plasma	68·2
(2) Serum—	
(*a*) Percentage of plasma	92·5
(*b*) Percentage of whole blood drawn . . .	**62·8**

TABLE VII.

HÆMORRHAGIC SEPTICÆMIA. DEFIBRINATION METHOD.

Hill Bulls. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	1,900	1,700	89·5	1,000	58·8	**52·6**
2	15,900	12,900	81·1	5,600	43·4	**35·2**
3	1,900	1,500	78·9	950	63·3	**50·0**
4	10,500	8,550	81·4	5,800	67·8	**55·2**
TOTAL	30,200	24,650	81·6	13,350	54·1	**44·2**

Average yields—

(1) Defibrinated blood	82·7
(2) Serum—	
(*a*) Percentage of defibrinated blood . . .	58·4
(*b*) Percentage of whole blood drawn . . .	**48·2**

TABLE VIII.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Hill Bulls. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	Serum		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	12,100	7,350	60·7	6,800	92·5	**56·2**
2	2,000	1,380	69·0	1,250	90·6	**62·5**
3	2,000	1,250	62·5	1,050	84·0	**52·5**
TOTAL	16,100	9,980	62·0	9,100	91·2	**56·5**

Average yields—	
(1) Plasma	64·1
(2) Serum—	
(*a*) Percentage of plasma	89·0
(*b*) Percentage of whole blood drawn . . .	**57·1**

TABLE IX.

HÆMORRHAGIC SEPTICÆMIA. DEFIBRINATION METHOD.

Hill Bulls. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	16,100	11,700	72·7	8,500	72·6	**52·7**

TABLE X.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Hill Bulls. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	SERUM		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	12,100	7,150	59·1	6,550	91·6	**54·1**
2	14,100	8,050	57·1	7,250	90·0	**51·4**
3	2,000	1,350	67·5	1,250	92·6	**62·5**
4	3,600	2,300	63·9	2,050	89·2	**57·2**
TOTAL .	31,800	18,850	61·9	17,100	90·8	**53·7**

Average yields—
- (1) Plasma . . . . 59·4
- (2) Serum—
  - (*a*) Percentage of plasma . . . . 90·8
  - (*b*) Percentage of whole blood taken . . . . **56·2**

TABLE XI.

HÆMORRHAGIC SEPTICÆMIA. OXALATE METHOD.

Hill Bulls. *3rd Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	SERUM		
				Volume obtained.	Percentage of plasma	Percentage of whole blood drawn
1	12,100	7,550	62·4	6,150	81·5	**50·8**
2	2,000	1,300	65·0	1,200	92·3	**60·0**
3	1,500	900	60·0	800	88·8	**53·3**
4	14,100	9,750	69·1	8,750	89·2	**61·7**
TOTAL .	29,700	19,500	65·6	16,850	86·4	**56·7**

Average yields—
- (1) Plasma . . . . 64·1
- (2) Serum—
  - (*a*) Percentage of plasma . . . . 88·0
  - (*b*) Percentage of whole blood drawn . . . . **56·4**

For the sake of ready comparison the results obtained with hæmorrhagic septicæmia serum are summarized in Table XII below:—

TABLE XII.

Hæmorrhagic Septicæmia. Serum yields.

Animal	Nature of bleeding	Average yield of serum. Percentage of whole blood drawn	
		Defibrination method	Oxalate method
	Bleedings.		
Buffaloes	1st	50·6	56·4
,,	2nd	52·0	59·2
,,	3rd	58·5	62·8
Hill Bulls	1st	48·3	57·1
,, ,,	2nd	52·7	56·2
,, ,,	3rd	..	56·4

TABLE XIII.

Anthrax. Defibrination method.

Buffaloes. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	1,900	1,600	84·2	900	56·2	**47·4**
2	12,100	10,400	85·9	5,900	56·7	**48·6**
3	2,200	1,850	84·1	1,400	75·7	**63·6**
4	11,100	9,350	84·2	3,650	39·0	**32·9**
5	23,900	19,650	82·2	10,250	52·2	**42·9**
6	1,500	1,400	93·3	800	57·2	**53·3**
7	1,900	1,600	84·2	1,000	62·5	**52·6**
8	15,000	12,800	85·3	6,800	53·1	**45·3**
Total .	69,600	58,650	84·3	30,700	52·3	**44·1**

Average yields—
(1) Defibrinated blood . . . . 85·4
(2) Serum—
(*a*) Percentage of defibrinated blood . . . . **56·6**
(*b*) Percentage of whole blood drawn . . . **48·6**

TABLE XIV.

ANTHRAX. OXALATE METHOD.

Buffaloes. *1st Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	Serum		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	6,500	3,950	60·8	3,620	91·6	**55·7**
2	3,200	1,730	53·0	1,520	87·9	**47·5**
3	19,000	11,350	59·7	10,250	90·3	**53·9**
4	5,100	2,900	56·9	2,700	93·1	**52·9**
5	5,100	3,500	68·6	3,250	92·9	**63·7**
6	3,400	2,500	73·5	2,300	92·0	**67·6**
7	7,400	4,450	60·1	3,900	87·6	**54·0**
8	13,700	9,200	67·1	8,100	88·0	**59·0**
9	14,500	9,500	65·5	8,050	84·7	**55·5**
10	13,600	8,700	64·0	7,750	89·1	**56·2**
TOTAL .	91,500	57,780	63·1	51,440	89·0	**56·2**

Average yields—

(1) Plasma . . . . 62·9

(2) Serum—

(*a*) Percentage of plasma . . . . . . 89·7

(*b*) Percentage of whole blood drawn . . . **56·6**

TABLE XV.

ANTHRAX. DEFIBRINATION METHOD.

Buffaloes. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	Serum		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	3,200	2,400	75·0	1,800	75·0	**56·2**
2	2,900	2,250	77·6	1,750	77·8	**60·3**
3	18,400	15,050	81·8	10,000	66·5	**54·4**
4	3,200	2,200	68·8	1,500	68·2	**46·9**
5	16,400	14,100	86·0	8,400	59·6	**51·2**
6	24,300	20,700	85·2	13,000	62·8	**53·5**
7	1,900	1,550	81·6	1,100	71·0	**57·9**
8	15,000	13,300	88·7	8,100	60·9	**54·0**
TOTAL .	85,300	71,550	83·9	45,650	63·8	**53·5**

Average yields—

(1) Defibrinated blood . . . . 80·6

(2) Serum—

(*a*) Percentage of defibrinated blood . . . 67·9

(*b*) Percentage of whole blood drawn . . . **54·3**

TABLE XVI.

ANTHRAX. OXALATE METHOD.

Buffaloes. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	SERUM		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	19,000	12,500	65·8	11,210	89·7	**59·0**
2	17,200	11,100	64·5	9,700	87·4	**56·4**
3	9,300	6,100	66·0	5,550	90·4	**59·7**
4	3,400	2,400	64·7	2,100	87·5	**61·1**
5	7,400	4,500	60·8	4,050	90·0	**53·3**
6	14,500	10,500	72·4	9,500	90·5	**65·5**
7	14,500	10,200	70·3	8,800	86·2	**60·6**
TOTAL .	85,500	57,300	67·0	50,910	88·8	**59·5**

Average yields—

(1) Plasma	66·6
(2) Serum—	
(*a*) Percentage of plasma	88·8
(*b*) Percentage of whole blood drawn . .	**59·4**

TABLE XVII.

ANTHRAX. DEFIBRINATION METHOD.

Buffaloes. *2nd Bleedings.*

Series	Volume of blood taken cc.	Volume of defibrinated blood obtained	Percentage yield of defibrinated blood	SERUM		
				Volume obtained	Percentage of defibrinated blood	Percentage of whole blood drawn
1	22,100	20,500	92·7	13,650	66·5	**61·70**

TABLE XVIII.

ANTHRAX. OXALATE METHOD.

Buffaloes. *3rd Bleedings.*

Series	Volume of blood taken cc.	Volume of plasma obtained	Percentage yield of plasma	Serum		
				Volume obtained	Percentage of plasma	Percentage of whole blood drawn
1	19,000	13,500	71·0	11,300	83·7	**59·5**
2	12,100	9,050	74·8	7,900	87·3	**65·3**
3	3,400	2,600	76·4	2,350	90·4	**69·1**
4	7,400	5,000	67·5	4,500	90·0	**60·8**
TOTAL .	41,900	30,150	72·0	26,050	86·4	**62·2**

Average yields—	
(1) Plasma	72·4
(2) Serum—	
(*a*) Percentage of plasma	87·9
(*b*) Percentage of whole blood drawn . . .	**63·7**

For comparison, the results obtained with anthrax serum are collected in Table XIX below :—

TABLE XIX.

ANTHRAX. SERUM YIELDS.

Animal	Nature of bleeding	AVERAGE YIELD OF SERUM PERCENTAGE OF WHOLE BLOOD DRAWN	
		Defibrination method	Oxalate method
Buffaloes	1st . . .	48·6	56·6
,, ...	2nd . . .	54·3	59.4
,, ...	3rd . .	61·7	63·7

An examination of Tables XII and XIX shows at a glance that in every case the oxalate method gives higher yields of serum than the defibrination method. In the latter process the figures in the previous tables show that a much greater loss of blood takes place during defibrination than is generally supposed. As regards the oxalate method it is interesting to observe the surprisingly small amount of serum lost in the clot, the final yield generally amounting to about 90 per cent. of the plasma. Tables XII and XIX also show the influence exerted on the yield by the nature of the bleeding. The increased yields exhibited by 2nd and 3rd bleedings as compared with 1st clearly indicate a change in the blood composition and a series of analyses is at present in progress to determine the extent of this change. At the same time the blood changes in animals in process of immunisation against anthrax and hæmorrhagic septicæmia are being investigated.

In the description of the oxalate method given above in this paper attention was drawn to a striking difference between the blood of buffaloes and of hill bulls. In the case of the former the corpuscles settle almost at once whereas with hill bull blood but little separation takes place even in twenty-four hours. This difference is exhibited by the blood of both normal and immunised animals. A series of analyses has therefore been carried out with normal animals to see whether there might be any marked analytical differences between the two bloods which would account for this behaviour. The results are given below in Table XX.

The analytical data investigated and the methods employed were as follows :—

(1) Count of red cells. This was kindly carried out for me by Dr. G. H. Macalister, Pathologist in this laboratory, to whom my thanks are due.

(2) Estimation of protein in (*a*) whole blood, (*b*) corpuscles, (*c*) plasma. The corpuscles from a definite volume of blood were washed on the centrifuge with saline until free from serum, etc.

(3) Calculation from (2) of the relative proportions of plasma and corpuscles in the blood.

(4) Analysis of serum proteins :—
  (*a*) Total.
  (*b*) Albumin and Globulin.
  (*c*) Salt-insoluble proteins.

Total protein. This was calculated from the nitrogen content estimated by Kjeldahl's method.

Albumin and Globulin. The globulin was separated by saturation with magnesium sulphate and the nitrogen in the filtrate estimated.

TABLE XX.

Comparison of Buffalo and Hill Bull blood.

Serial number	Animal	Red cell count, Millions per cc.	WHOLE BLOOD	CORPUSCLES	PLASMA		SERUM						
			Protein in 10 cc. grms.	Protein in corpuscles from 10 cc. blood grms.	Protein in 10 cc. grms.	Volume of plasma in 10 cc. blood.	Total protein in 10 cc. grms.	Albumin in 10 cc. grms.	Globulin in 10 cc. grms.	Salt in soluble protein in 10 cc. grms.	Percentage of Albumin.	Percentage of Globulin.	Percentage of Salt-insoluble protein.
1	Buffalo	5·60	1·7650	1·2984	0·7219	64·6	0·6620	0·2756	0·3864	0·0471	41·6	58·4	7·2
2	,, ...	5·35	1·7085	1·1813	0·7815	73·0	0·7284	0·3139	0·4145	0·2450	43·1	56·9	33·6
3	,, ...	6·40	1·8230	1·2480	0·7937	72·4	0·7405	0·3380	0·4025	0·0974	45·6	54·4	13·2
4	,, ...	6·10	1·7872	1·3200	0·7260	64·3	0·6695	0·3128	0·3567	0·0722	46·7	53·3	10·8
5	,, ...	6·50	1·8342	1·3016	0·7613	69·9	0·7066	0·2931	0·4135	0·1258	41·4	58·5	17·8
6	,, ...	5·80	1·5903	1·1189	0·7033	67·0	0·6355	0·2669	0·3686	0·0514	42·0	58·0	8·1
7	,, ...	6·10	1·7992	1·3169	0·7218	67·0	0·6639	0·2855	0·3784	0·0591	43·0	57·0	8·9
8	Hill Bull	5·52	1·7850	1·2812	0·8090	62·2	0·7615	0·2735	0·4880	0·1520	35·9	64·0	19·9
9	,, ,, ...	4·72	1·6355	1·1233	0·7525	68·0	0·7284	0·2726	0·4558	0·1925	37·4	62·6	26·4
10	,, ,, ...	5·70	1·8390	1·2398	0·9078	66·0	0·9100	0·2986	0·6114	0·2767	32·9	67·1	30·4
11	,, ,, ...	5·32	1·5805	1·1058	0·6639	71·5	0·6355	0·2177	0·4178	0·0930	34·3	65·7	14·6
12	,, ,, ...	5·60	1·6986	1·1539	0·7941	67·4	0·7536	0·2428	0·5108	0·1531	32·3	67·7	20·3
13	,, ,, ...	5·07	1·5673	1·0259	0·7525	71·9	0·7120	0·2570	0·4550	0·1444	36·1	63·9	20·0
14	,, ,, ...	5·10	1·5695	1·1102	0·6650	68·1	0·6202	0·2286	0·3917	0·4703	36·8	63·2	7·6

Salt-insoluble proteins. These were removed by saturation with salt and estimations of the residual nitrogen in the filtrate then made.

All analyses were carried out in duplicate. The figures given on previous page, Table XX, are the mean results of closely agreeing experiments.

On examining the above figures it will be seen that there is practically no difference in the percentage of plasma in the two bloods, the average figures being 68·3 and 67·8 for buffaloes and hill bulls respectively. When one comes to the serum, however, a marked difference is at once apparent, the hill bull serum in every case containing considerably more globulin than buffalo serum. The average result expressed in percentages of the total serum protein was 56·6 for buffaloes and 65·1 for hill bulls. It is obvious that a change of this pronounced nature might very easily have a great influence on the physical properties of the blood. It has unfortunately been impossible at the present time to carry out any viscosity determinations but it seems likely that the difference in behaviour of the two bloods is due to this factor. Another interesting point is the extreme irregularity of the salt-insoluble protein content, a fact previously noticed by other observers. Meantime for convenience in working it is obviously desirable to employ as large a percentage of buffaloes as possible as in this way the volume of blood to be centrifugalised can be reduced to a minimum.

SUMMARY AND CONCLUSIONS.

(1) In the preparation of hæmorrhagic septicæmia and anthrax sera the oxalate method gives a considerably increased yield of serum as compared with the defibrination method.

(2) The serum prepared by the oxalate method is greatly superior in appearance to that obtained by defibrination and centrifugalisation.

(3) In the case of buffalo blood the oxalate method saves 50 per cent. of the centrifugalising as the corpuscles settle rapidly and the plasma can be syphoned off. This point is of considerable practical importance where large quantities of sera are dealt with as the consequent economy in engine power entails an appreciable saving of expense.

(4) With hill bull blood the corpuscles show little tendency to settle and the whole of the blood has to be centrifugalised as in the defibrination method.

(5) In both hæmorrhagic septicæmia and anthrax bleedings if a series of bleedings be taken at short intervals the yield of serum increases progressively, the third bleeding giving a larger percentage of serum than the second which in turn yields more than the first.

(6) Analyses of normal buffalo and hill bull blood show that the serum from the latter animal contains a considerably larger percentage of globulins than does that from buffaloes. This seems to be the chief point of difference between the two bloods.

In conclusion I wish to acknowledge the able assistance rendered me by Mr. G. P. Goffi, 2nd Laboratory Assistant. The misadventures so frequently consequent on the introduction of a new working method were almost entirely avoided owing to the careful way in which he supervised every stage of the process.

THE IMPERIAL BACTERIOLOGICAL LABORATORY,

MUKTESAR, UNITED PROVINCES.

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